Apoptosis and change of competence limit the size of the vulva equivalence group in *Pristionchus pacificus*: a genetic analysis

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Background: To understand how alterations in the molecular mechanisms underlying developmental processes generate a diversity of biological forms, comparative developmental biology can be combined with genetic analysis. The formation of the nematode vulva is one tractable system for such evolutionary developmental analysis, as much is understood about its development in *Caenorhabditis elegans*. In *Caenorhabditis*, six of twelve ventral epidermal cells form the 'vulva equivalence group'; although all six cells are competent to adopt vulval cell fates in response to an inductive signal, only three of these cells are induced to form vulval tissue.

Results: In some species of the nematode families Rhabditidae, Neodiplogastridae and Panagrolaimidae, the number of cells in the vulva equivalence group is limited by apoptosis and decreased responsiveness to inductive signals (competence). We have initiated a genetic analysis in one of these species, *Pristionchus pacificus*, to understand the evolution of the specification of ventral epidermal cells that are competent to generate the vulva. A *ped-4* mutation restores competence to an incompetent cell. Mutation of either of two other genes of *Pristionchus* cause two anterior cells that die in wild-type to survive. A *ped-5* mutation causes these cells to be competent to respond to inductive signals, expanding the equivalence group. A *ped-6* mutation causes these cells to form ectopic, anterior vulva-like invaginations.

Conclusions: During nematode evolution, apoptosis and change of competence alter the number and potency of ventral epidermal cells. The phenotypes of *Pristionchus* mutants suggest that alterations in homeotic gene control of anteroposterior patterning is involved in creating this cellular diversity.

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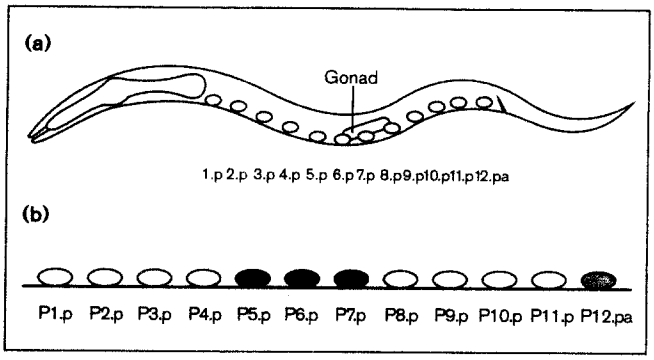
Background

There are striking similarities at the molecular level among developmental processes throughout the animal kingdom, as well as among different aspects of development within one organism. This universality of mechanism stands in contrast to the broad diversity of biological form at the organismic level. From this discrepancy arises the question of how developmental processes evolve. To address this question at the level of single cells, we need to understand how similar underlying genetic components generate cells in different states. This genetic control of cell specification can be examined by combining comparative developmental biology with genetic analysis. Such comparative developmental genetics is possible in the case of the development of the vulva in *Caenorhabditis elegans* and other nematodes.

The vulva of *Caenorhabditis* is a derivative of the ventral epidermis, which consists of twelve epidermal cells, P(1–12).p, generally called Pn.p cells (Fig. 1) [1]. These cells are equally distributed between pharynx and anus, and are named according to their anteroposterior position (Fig. 1a). The anterior cells P(1,2).p and the posterior cells P(9–11).p form part of the syncytial epidermis (Fig. 1b). The posterior cell P12.p divides, and the surviving daughter cell P12.pa, also called hyp12, forms part of the rectum (Fig. 1b). In the central body region, the *Hom-C* gene *lin-39* specifies the six cells P(3–8).p as vulva precursor cells (VPCs), the equivalence group for vulva formation [2–4]. Three of these cells, P(5–7).p, are induced by the gonadal anchor cell (AC) to form vulval tissue [5]. P6.p, the cell closest to the AC, has the 1° cell fate, generating eight progeny (Fig. 1b). The two more distal cells, P(5,7).p have the 2° cell fate, each generating seven progeny (Fig. 1b). The other three VPCs, P(3,4,8).p, form non-specialized epidermis, the 3° cell fate (Fig. 1b). Genetic and molecular analysis in *Caenorhabditis* indicates that three intercellular signals — an inductive signal, a lateral signal and a negative signal — are involved in cell-fate specification of the VPCs [6].

Figure 1



(a) Schematic summary of the position of the Pn.p cells and P12.pa in the first stage juvenile of *Caenorhabditis elegans*. **(b)** Position-specific cell fates of the eleven ventral Pn.p cells and the P12.pa cell in *Caenorhabditis elegans*. P(1,2,9,10,11).p (white ovals) fuse with the hypodermal syncytium hyp7 in the L1 stage. P12.pa (light blue oval) forms hyp12, a cell involved in the regulation of the rectum. The cells of the vulva equivalence group divide during the L3 stage. Cells having a 3° fate (yellow ovals) fuse with the syncytial hypodermis, whereas the cells that adopt vulval fates (blue oval, 1° fate; red ovals, 2° fate) undergo a morphogenetic process during the L4 stage.

Here, we report on the comparative analysis of vulva development in members of three distinct nematode families. Cell-lineage analysis indicates that apoptosis and a change in responsiveness to inductive signals (competence) alter the size of the vulva equivalence group in nematodes. In one species, *Pristionchus pacificus*, we have started to use genetic analysis to investigate the evolution of cell-fate specification of ventral epidermal cells. We have identified two mutations, *ped-5* and *ped-6*, which cause two anterior cells that die in wild-type to survive. In *ped-5* mutant animals, these cells expand the vulva equivalence group in the anterior. In *ped-6* mutant animals, these cells form ectopic anterior vulva-like structures, resembling the phenotype of multivulva mutants described in *Caenorhabditis* [6]. A third mutation, *ped-4*, restores competence to an incompetent cell, but does not change which cells die.

Results

Apoptosis changes the number of Pn.p cells in nematode evolution

A survey of vulva formation within the Rhabditidae family of nematodes, which includes *Caenorhabditis* [7,8], has shown that the vulva equivalence group consists of six (P(3–8).p) or five (P(4–8).p) cells that are recruited as a subset of the eleven Pn.p cells described for *Caenorhabditis* [9,10]. We have expanded this comparison to one additional genus of the Rhabditidae, and to species of the Panagrolaimidae and Neodiplogastridae families, and have identified species with four instead of eleven epidermal Pn.p cells (Table 1). Cell-lineage analysis revealed that, in females or hermaphrodites of *Poikilolaimus oxycerca* (Rhabditidae), *Panagrolaimus* sp., PS 1159 (Panagrolaimidae) and *Pristionchus pacificus* (Neodiplogastridae), the remaining epidermal cells were the four central cells P(5–8).p and the equivalent of the *Caenorhabditis* hyp12 cell. In *Poikilolaimus*, P(1–4).p and P(9–11).p underwent programmed cell death in the first larval stage (L1) (Table 1). In *Panagrolaimus* sp. PS 1159, P(1–4).p died, whereas P(9–11).p survived (Table 1). Late in the L1 stage, P(9–11).p moved to a dorsal position in the ventral epidermis and lost the typical morphology of epidermal cells. Later on, these cells were indistinguishable from the surrounding neurons, such that only five of the eight surviving cells, P(5–8).p and P12.pa, had an epidermal appearance. In *Pristionchus*, P(1–4).p and P(9–11).p died prior to hatching (Table 1).

The change of fate of P(1–4).p and P(9–11).p in these three species therefore limits the size of a potential vulva equivalence group to the four central cells, P(5–8).p. The finding that, in species of three distinct families of nematodes, only P(5–8).p and hyp12 adopt an epidermal or vulval fate, suggests that phenotypically similar, but phylogenetically independent, transformations occurred. It remains unclear whether the twelve or five epidermal cell pattern is evolutionarily original, and thus we cannot infer the direction of the observed evolutionary transformations.

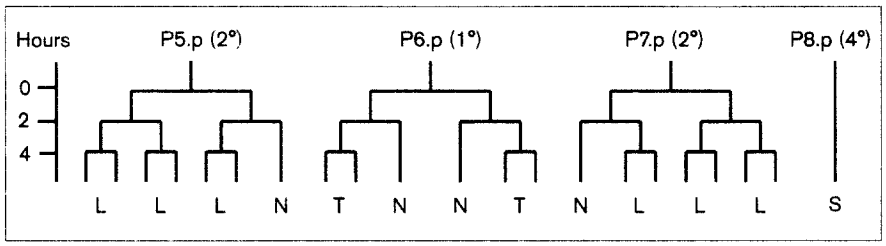
Table 1

Summary of the Pn.p cell fates in *Caenorhabditis, Poikilolaimus, Panagrolaimus* and *Pristionchus*.

Species	Pn.p formation	P1.p	P2.p	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	P9.p	P10.p	P11.p	P12.pa
Caenorhabditis elegans	L1	S	S	E	E	E	E	E	E	S	S	S	hyp12
Poikilolaimus oxycerca	L1	X	X	X	X	E	E	E	E	X	X	X	hyp12
Panagrolaimus sp. PS 1159	L1	X	X	X	X	E	E	E	E	N	N	N	hyp12
Pristionchus pacificus	Embryo	X	X	X	X	E	E	E	S	X	X	X	hyp12

The Pn.p cells form in the L1 stage in *Caenorhabditis, Poikilolaimus* and *Panagrolaimus*, whereas they form during embryogenesis in *Pristionchus*. In *Caenorhabditis*, P(3–8).p are VPCs (E), whereas P(1,2).p and P(9–11).p fuse with the epidermal syncytium hyp7 (S). The P12 derivative (P12.pa) is a special cell, hyp12. In addition to hyp12, only four epidermal Pn.p cells are present in the three analyzed species. P(5–8).p are VPCs in *Poikilolaimus* and *Panagrolaimus*, whereas only P(5–7).p are VPCs in *Pristionchus* (see Fig. 2 for details). E, VPC; S, fusion with the hypodermis; X, programmed cell death; N, cell with neuronal morphology. Cells that form vulval tissue in wild-type are underlined. The vulva is formed by four VPCs in *Panagrolaimus* and by three VPCs in the other species.

Figure 2



The vulva cell lineage in *Pristionchus pacificus*. P6.p has the 1° lineage forming six progeny. P(5,7).p have the 2° lineage, generating seven progeny. P8.p does not divide. We refer to the first VPC division as 0 hour. Abbreviations: T, transverse division; L, longitudinal division; N, no division (according to the convention used for *Caenorhabditis* [4]).

While we speculate that the twelve cell condition is ancestral, reversions that do not interfere with the major derivatives of the Pn.p cells, the vulva and hyp12, could occur frequently.

Change of competence further restricts the vulva equivalence group in *Pristionchus*

In *Poikilolaimus* and *Panagrolaimus*, the four remaining central cells were VPCs — all cells adopted or could adopt vulval cell fates. The vulva in *Panagrolaimus* was formed by P(5–8).p. P(5,8).p had the 2° cell fate, and P(6,7).p had the 1° cell fate, with a cell lineage identical to that of *Panagrellus redivivus* [11]. In *Poikilolaimus*, P(5–7).p formed the vulva, whereas P8.p did not divide. However, when we ablated P7.p, P8.p formed part of the vulva in four of the six animals analyzed; this cell was therefore competent to respond to inductive signalling if given the opportunity.

In *Pristionchus*, however, an additional difference of competence further restricted the equivalence group. The vulva was formed by the progeny of P(5–7).p (Figs. 2, 3a,b and 4a), and was induced by the gonad (Table 2). Ablation experiments indicated that P8.p, which did not divide in wild-type animals, was not a VPC capable of responding to inductive signal. Isolated P8.p cells, or P8.p in the presence of other cells, never formed vulval tissue (Table 2). P8.p was not a member of the vulva equivalence group (a '4° cell fate'), and therefore there are only three VPCs in *Pristionchus*; these form the vulva in the intact wild-type animal. We conclude that two different cellular mechanisms, apoptosis and change of competence, limit the size of the vulva equivalence group in *Pristionchus*.

Although isolated P5.p or P6.p cells could have the 1° cell fate, a single P7.p generated 2° or 3° cell fates, suggesting that P7.p was intrinsically different. However, the migration of P7.p towards the AC was delayed — in most animals, P7.p started to divide before it reached the AC region. P7.p might be too far away from the source of inductive signal when induction occurs. Thus, we cannot rule out the possibility that P7.p is different from P(5,6).p with respect to cell migration, but is equivalent with respect to competence. While a similar argument can be made for the incompetence of P8.p, our genetic analysis described below suggests that P8.p is different.

Mutants of *Pristionchus* have surviving anterior cells that die in wild-type

To analyze the genetic control of Pn.p cell specification in *Pristionchus*, we isolated mutants with defective Pn.p cell determination. Genetic analysis in *Pristionchus* was carried out using the strategy described for *Caenorhabditis* [12]. From a screen of 6400 gametes, we examined the anatomy of 100 mutants that were defective in egg-laying, and isolated 15 mutants with abnormal cell lineages in the ventral epidermis [13]. Three vulvaless mutants were isolated in which P(5–8).p did not divide; the phenotype of these mutants resembled that of gonad-ablated wild-type animals (data not shown). In most of the other mutants, the number of Pn.p cells in the ventral epidermis changed with respect to wild type; we call these mutants Ped, for P ectoblast determination. Here, we describe three Ped mutant strains, each of which carried a recessive, single-locus mutation (based on segregation after a backcross to wild-type). Complementation tests revealed that these mutations were in different genes (Table 3).

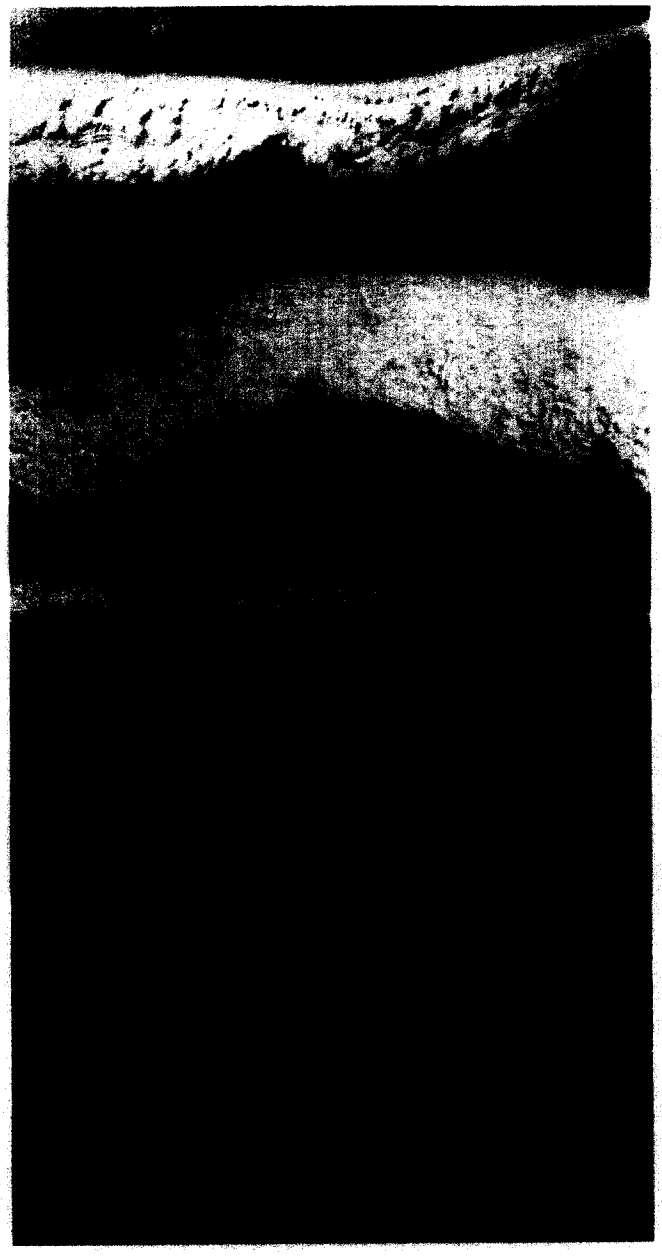
Table 2

Ablation experiments in *Pristionchus pacificus*.

	P5.p	P6.p	P7.p	P8.p	Number of animals
Wild-type	2°	1°	2°	4°	Many
Z(1,4)⁻	3°	3°	3°	4°	10/10
P(5–7).p⁻	–	–	–	4°	7/7
P(6–8).p⁻	1°	–	–	–	8/11
	2°	–	–	–	3/11
P(5,7,8).p⁻	–	1°	–	–	5/5
P(5,6).p⁻	–	–	2°	4°	6/8
	–	–	3°	4°	2/8

Cells were ablated in the Pn.p stage shortly after hatching, using standard technology as described [4,10]. Z(1,4)⁻, ablation of gonadal precursor cells; dash (–), ablation of a particular VPC. Each line in the table represents one type of experiment. See text for details.

Figure 3



Nomarski photomicrographs of lateral views of *Pristionchus pacificus* hermaphrodites. **(a)** Wild-type animal before the onset of vulval cell divisions, showing the four cells P(5–8).p. **(b)** Wild-type animal after the vulval cell divisions are completed, showing the seven progeny of P5.p and P7.p, the cells with the 2° cell fate. Progeny of P6.p are not visible in this plane of focus. AC, anchor cell. The undivided cell, P8.p, is posterior to the vulva. **(c)** A *ped-5* mutant animal with a vulva (V) formed by P(5–7).p and the surviving cells P(3,4).p. **(d)** A *ped-6* mutant animal with a vulva (V) formed by P(5–7).p and a second invagination formed by P4.p anteriorly. P3.p did not divide in this animal. Scale bar, 20 μm.

In *ped-5(sy344)* mutant animals, P(3,4).p (which died in wild-type *Pristionchus*) survived but did not divide later in development (Figs 3c and 4b). To determine whether these cells were differentiated epidermal cells (4° fate, like P8.p) or VPCs (3° fate, like uninduced VPCs), we carried out a series of VPC ablation experiments and found that P(3,4).p were VPCs (Fig. 4b). After ablation of P5.p or P6.p, P4.p had the 2° cell fate in most animals (8/9 and 11/13, respectively). If P(4,5).p were both ablated, P3.p could also form part of the vulva (2/10 animals). This low frequency might reflect the more anterior position of P3.p, consistent with results obtained in *Caenorhabditis* [4]. If P(5,6).p were ablated, a normal vulva could be formed by P(3,4,7).p, with P4.p having the 1° cell fate (2/12 animals) (Fig. 4b). The *ped-5* mutation therefore expands the vulva equivalence group in the anterior region. Other apoptotic cell deaths of ventral epidermal and neuronal cells of the Pn lineages were unaffected in *ped-5* mutant animals, suggesting that they were not generally defective in apoptosis. The egg-laying defective phenotype of *ped-5* was not due to the described changes in the ventral epidermis, but rather a result of abnormal development of the gonad.

In *ped-6(sy345)* mutant animals, P(3,4).p survived and differentiated to generate vulva-like structures with one or two additional anterior invaginations (Figs 3d and 4c). The cell lineage of P(5–7).p was not affected in intact *ped-6* animals, but after ablation of the gonad, P(3,4).p and P(5–7).p differentiated to form three to four vulva-like structures (5/5 animals) (Fig. 4c). We ruled out the possibility that P(3,4).p signal their neighbours to form vulval tissue by demonstrating that P(5–7).p still generated vulva-like structures in the absence of P(3,4).p and the gonad (5/5 animals) (Fig. 4c). In *Caenorhabditis*, gonad-independent vulva differentiation occurs in multivulva mutants, such as those carrying mutations that activate *let-60 ras* [6]. The *ped-6* mutation is therefore phenotypically similar to a multivulva mutation, but affects not only the normal VPCs but also the two anterior cells, P(3,4).p, which die in wild-type. P8.p was not affected in *ped-6* mutants, indicating its incompetence to form vulval tissue. As in *ped-5* mutants, apoptosis of other ventral epidermal and neuronal cells was unaltered in *ped-6* mutants, and the egg-laying defective phenotype was caused by abnormal development in tissues other than the epidermis.

In *ped-4(sy346)* mutant animals, P8.p could differentiate and form vulva-like structures (Fig. 4d). Anatomical observation indicated that P8.p generated 2°-like lineages in ~ 40 % of *ped-4* animals (12/27). Moreover, in ~ 10 % of *ped-4* animals (3/27), P7.p generated a 2°-like lineage but its progeny did not connect to the progeny of its neighbour P6.p, resulting in an independent, smaller invagination. In gonad-ablated animals, P7.p or P8.p or both could generate vulval cells (Fig. 4d). After ablation of the gonad and P(5–7).p or P(5,6,8).p, the remaining cell could still generate vulval tissue (Fig. 4d). The *ped-4* mutation therefore affects the competence of both P7.p and P8.p, but this is not due to signals from their neighbouring Pn.p cells.

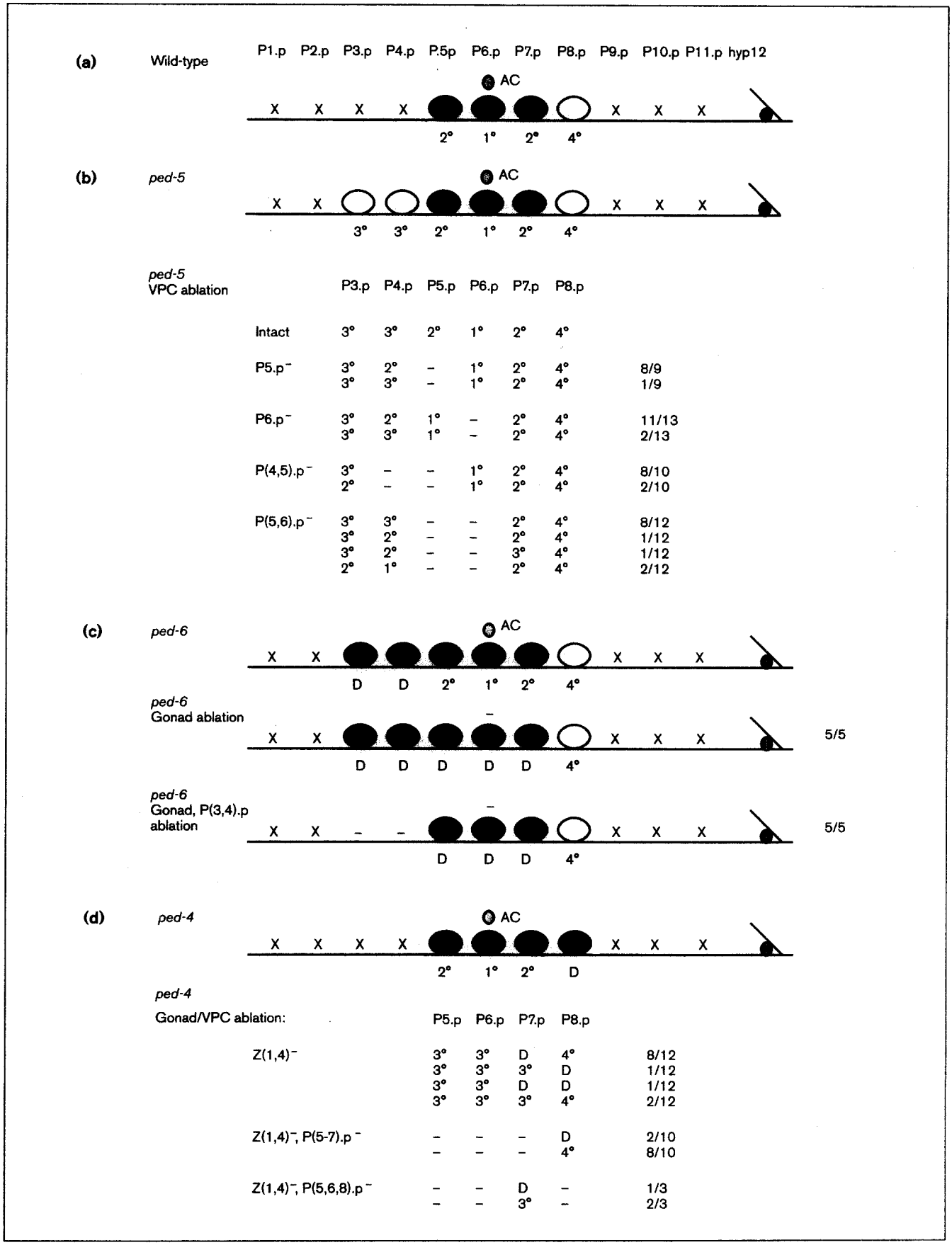

(a)
Wild-type
P1.p P2.p P3.p P4.p P5.p P6.p P7.p P8.p P9.p P10.p P11.p hyp12
AC
X X X X 2° 1° 2° 4° X X X
(b)
ped-5
AC
X X 3° 3° 2° 1° 2° 4° X X X
ped-5
VPC ablation
P3.p P4.p P5.p P6.p P7.p P8.p
Intact 3° 3° 2° 1° 2° 4°
P5.p⁻ 3° 2° – 1° 2° 4° 8/9
3° 3° – 1° 2° 4° 1/9
P6.p⁻ 3° 2° 1° – 2° 4° 11/13
3° 3° 1° – 2° 4° 2/13
P(4,5).p⁻ 3° – – 1° 2° 4° 8/10
2° – – 1° 2° 4° 2/10
P(5,6).p⁻ 3° 3° – – 2° 4° 8/12
3° 2° – – 2° 4° 1/12
3° 2° – – 3° 4° 1/12
2° 1° – – 2° 4° 2/12
(c)
ped-6
AC
X X D D 2° 1° 2° 4° X X X
ped-6
Gonad ablation
X X D D D D D 4° X X X 5/5
ped-6
Gonad, P(3,4).p
ablation
X X – – D D D 4° X X X 5/5
(d)
ped-4
AC
X X X X 2° 1° 2° D X X X
ped-4
Gonad/VPC ablation:
P5.p P6.p P7.p P8.p
Z(1,4)⁻ 3° 3° D 4° 8/12
3° 3° 3° D 1/12
3° 3° D D 1/12
3° 3° 3° 4° 2/12
Z(1,4)⁻, P(5-7).p⁻ – – – D 2/10
– – – 4° 8/10
Z(1,4)⁻, P(5,6,8).p⁻ – – D – 1/3
– – 3° – 2/3

Figure 4 (facing page)

Schematic summary of persistence, position and fates of the Pn.p cells and P12.pa in the ventral cord of *Pristionchus pacificus*. Abbreviations: X, programmed cell death; dash (–), ablation of the gonad or particular Pn.p cells; 1°, generates the lineage of wild-type P6.p; 2°, generates the lineage of wild-type P5.p or P7.p; 3° does not divide, but has been demonstrate to adopt 1° or 2° fates under experimental conditions; 4°, does not divide, but is apparently unable to adopt 1° or 2° fates; D, cells in mutants which adopt 2°-type lineages or hybrid lineages and form vulva-like structures. **(a)** In a wild-type animal, the vulva is formed by P(5–7).p (dark blue oval for 1° cell fate; red ovals for 2° cell fate). P8.p is not a VPC (yellow oval). P12.pa forms hyp12 (light blue circle). The other cells undergo programmed cell death. **(b)** In *ped-5(sy344)* mutant animals, P(3–4).p survive but do not undergo cell divisions later in development. Ablation of different combinations of VPCs indicate that P3.p and P4.p are VPCs. See text for details. **(c)** In *ped-6(sy345)* mutant animals, P(3–4).p survive and form vulva-like structures in the anterior (pink ovals). After ablation of the gonad, P(3–7).p differentiate. After ablation of the gonad and P(3,4).p together, P(5–7).p still form vulva-like structures, indicating that their differentiation is independent of P(3,4).p. **(d)** In *ped-4(sy346)* mutant animals, P8.p can form vulva-like structures. In some animals also P7.p forms an independent invagination. Ablation of the gonad and/or VPCs indicate that P(7,8).p differentiate independently of the AC and neighbouring VPCs.

Discussion

Our analysis of *Pristionchus* indicates that the generation and specification of the vulva equivalence group has changed during evolution among nematode families. In *Caenorhabditis*, these aspects of development involve the *Hom-C* gene *lin-39* [2,3]. Assuming that anteroposterior positional specification in *Pristionchus* also involves *Hom-C* genes, two models could account for the observed evolutionary and genetic transformations (Fig. 5). In the first model, *lin-39* is expressed only in the region of P(5–8).p (Fig. 5a). In this case, Pn.p cells receiving positional information from *lin-39* would survive, and all others would undergo programmed cell death. Expanding the *lin-39* expression domain towards P(3,4).p could explain the expansion of the equivalence group in *ped-5* mutants. However, this model cannot explain the multivulva mutation *ped-6*. In *Caenorhabditis*, multivulva mutations affect all cells of the *lin-39* expression domain [14], whereas this first model proposes that *ped-6* causes AC-independent differentiation of cells both inside and outside of this expression domain.

We therefore favour a model in which *lin-39* is expressed in the region of P(3–8).p, as in *Caenorhabditis* (Fig. 5b). However, additional positional information in P(3,4).p (for example, a more anterior *Hox* gene) might override the effect of *lin-39* in these cells. The *ped-5* mutation could be a reduction-of-function mutation in such an anterior *Hox* gene, and the LIN-39 protein might be dominant under these mutant circumstances, affecting P(3,4).p but not P(1,2).p. We speculate that the vulval equivalence group in *Pristionchus* results from a phylogenetic restriction; in this view, the *ped-5* mutation is a classic homeotic mutation [15].

We speculate that the multivulva mutation *ped-6* affects a negative regulator of vulva development, whose absence overrides the early positional information conferred by LIN-39. As P8.p is unaffected by these mutations, its competence may be, to some extent, under separate genetic control from the other Pn.p cells. In *Caenorhabditis*, P3.p is not always competent to form vulval cells, yet multivulva mutations prevent early fusion of P3.p with hyp7 syncytial epidermis; thus, anterior cells might be more sensitive to multivulva mutations than posterior cells [1,16,17].

We have provided evidence that P7.p and P8.p differ in competence with respect to P5.p and P6.p. In addition,

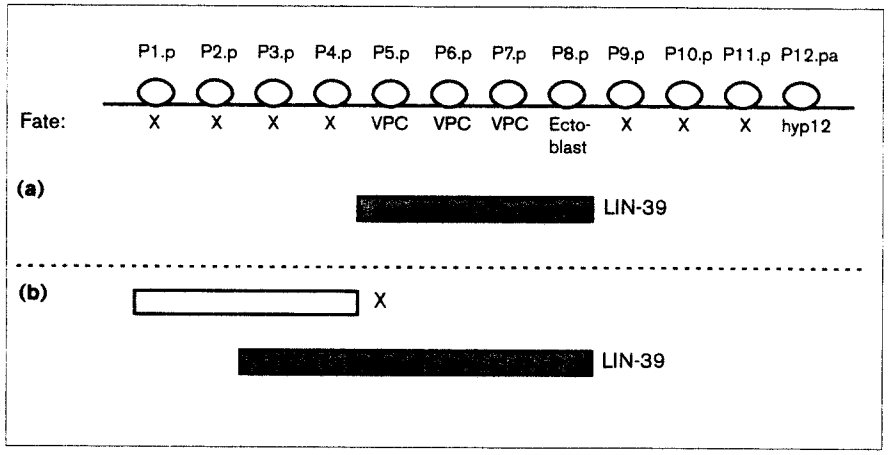
Table 3

Complementation analysis of the described mutants in *Pristionchus pacificus*.

♀ \ ♂	*ped-4(sy346)* +	*ped-5(sy344)* +	*ped-6(sy345)* +	± +
ped-4(sy346)* ; *unc1(sy306) ***ped-4(sy346) unc1(sy306)***	–	7/7 non-Ped, non-Egl	21/21 non-Ped, non-Egl	20/20 non-Ped, non-Egl
ped-5(sy344)* ; *dpy7(sy373) ***ped-5(sy344) dpy7(sy373)***	38/38 non-Ped, non-Egl	–	50/50 non-Ped, non-Egl	20/20 non-Ped, non-Egl
ped-6(sy345)* ; *dpy12(sy349) ***ped-6(sy345) dpy12(sy349)***	20/20 non-Ped, non-Egl	23/23 non-Ped, non-Egl	–	20/20 non-Ped, non-Egl

To distinguish self- from cross-progeny, we marked hermaphrodites with an unlinked recessive visible mutation (*m*), conferring either a Dumpy (Dpy) or an uncoordinated (Unc) phenotype. Cross progeny from a mating of such marked *ped* homozygous hermaphrodites with males heterozygous for a *ped* mutation would be wild-type for the visible marker phenotype (being *m*/+). All *ped* phenotypes are recessive. The *ped* mutants complement for the egg-laying defects (Egl) and for their Ped phenotype under Nomarski optics. The number of cross-progeny examined is indicated in the table.

Figure 5



Two models for cell fate specification in the ventral cord in *Pristionchus*. **(a)** According to this model, LIN-39 is only expressed in the region of P(5–8).p and might be responsible for the survival of the corresponding cells. **(b)** According to the second model, LIN-39 is expressed in the region of P(3–8).p. The phenotypic outcome is influenced by a gene product, X, which is expressed in P(1–4).p or, alternatively, only in P(3,4).p. We hypothesize that the factor X overrides the developmental decision of LIN-39 in P(3,4).p.

P7.p and P8.p differ from one another in their competence to adopt vulval fates. Two different mechanisms could explain these results. Firstly, homeotic control of P(7,8).p could specifically create a positional difference from P(5,6).p, without distinguishing P7.p and P8.p; P8.p could then be under the genetic control of other factors that cause its incompetence. Secondly, one process could control all of the differences in competence among P(5–8).p, and P(7,8).p could be differently influenced by the graded distribution of a homeotic control gene or a downstream target of *Hom-C* genes. In *Caenorhabditis*, the *Hom-C* genes *lin-39* and *mab-5* are coexpressed in the region of P(7,8).p, which could create a positional difference from the region of P(5,6).p, where only *lin-39* is expressed [2]. Based upon the *ped-4* mutant phenotype, in which both P7.p and P8.p are competent to form vulval tissue, we favour the second possibility.

These data provide the first examples of programmed cell death of Pn.p cells in a wild-type nematode. In *Caenorhabditis*, the gain-of-function mutants *lin-24* and *lin-33* have Pn.p cell deaths [14,18], and it is conceivable that in *Pristionchus*, *Panagrolaimus* and *Poikilolaimus*, these or similar genes are under control of position-specific control elements. Another possibility stems from the observation that, in *Caenorhabditis*, apoptosis of close relatives of the Pn.p cells, the Pn.aap and Pn.aaap cells, is controlled by the *Hom-C* genes [2]. Activation of the death program in Pn.p cells in *Pristionchus* might occur by alteration of this pre-existing machinery within the Pn lineages [1,11,19].

Conclusions

By analyzing evolutionary changes in the development of the vulva among species of three families of nematodes we have observed phylogenetically independent, but phenotypically similar, evolutionary transformations. Two different cellular mechanisms, apoptosis and change of competence, limit the size of the vulva equivalence group and create cellular diversity during evolution. In *Pristionchus*, we have combined a comparative developmental approach with genetic analysis, and have observed mutant phenotypes which suggest that alterations in the homeotic gene control of anteroposterior patterning is involved in creating these evolutionary differences.

Materials and methods

Nomenclature and strains

The strains used are from the Caltech nematode collection, kindly provided by L.K. Carta. We refer to a particular species to by the genus name. *Poikilolaimus oxycerca* is a male-female strain and was obtained from W. Sudhaus *via* D. Fitch. *Panagrolaimus* n. sp. PS 1159 is a new species, collected and analyzed by L.K. Carta (personal communication). The morphology, genetics and molecular biology of *Pristionchus pacificus* has been described recently [13]; it is a hermaphroditic species, has a three-day generation time and is easily cultured. All species were grown on lawns of *Escherichia coli* OP50 at 20 °C, as described for *Caenorhabditis* [12].

Cell lineage

For long-term observation, we used the technique of Sulston and Horvitz [1]. Nematodes were observed using a Zeiss microscope with a plan 100 objective and Nomarski interference contrast optics. Cell lineages were determined by continuous observation of nuclei as they divided. Complete lineages were observed in at least three animals.

Laser microsurgery

Ablation experiments were done according to the method of Sulston and White [16], using a Laser Science dye laser of the type described by Avery and Horvitz [20]. Animals were picked into 2.5 µl S Basal placed on a pad of 5 % agar in water containing 10 mM sodium azide as anaesthetic.

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References

1. Sulston JE, Horvitz HR: **Postembryonic cell lineage of the nematode *Caenorhabditis elegans*.** *Dev Biol* 1977, **56**:110–156.

2. Wang BB, Mueller-Immergluck MM, Austin J, Robinson NT, Chisholm A, Kenyon C: **A homeotic gene cluster patterns the anteroposterior body axis in *C. elegans*.** *Cell* 1993, **74**:29–42.
3. Clark SG, Chisholm AD, Horvitz HR: **Control of cell fates in the central body region of *C. elegans* by the homeobox gene *lin-39*.** *Cell* 1993, **74**:43–55.
4. Sternberg PW, Horvitz HR: **Pattern formation during vulval development in *C. elegans*.** *Cell* 1986, **44**:761–772.
5. Kimble J: **Lineage alterations after ablation of cells in the somatic gonad of *Caenorhabditis elegans*.** *Dev Biol* 1981, **87**:286–300.
6. Sternberg PW: **Intercellular signaling and signal transduction in *C. elegans*.** *Annu Rev Genet* 1993, **27**:497–521.
7. Sudhaus W: **Vergleichende Untersuchungen zur Phylogenetik, Systematik, Ökologie, Biologie und Ethologie der Rhabditidae (Nematoda).** *Zoologica* 1976, **43**, No. 125.
8. Andrassy I: Klasse *Nematoda*. Stuttgart: Fischer Verlag; 1984.
9. Sommer RJ, Sternberg PW: **Changes of induction and competence during the evolution of vulva development in nematodes.** *Science* 1994, **265**:114–118.
10. Sommer RJ, Sternberg PW: **Evolution of cell lineage and pattern formation in the vulval equivalence group of rhabditid nematodes.** *Dev Biol* 1995, **167**:61–74.
11. Sternberg PW, Horvitz HR: **Postembryonic nongonadal cell lineages of the nematode *Panagrellus redivivus*: description and comparison with those of *Caenorhabditis elegans*.** *Dev Biol* 1982, **93**:181–205.
12. Brenner S: **The genetics of *Caenorhabditis elegans*.** *Genetics* 1974, **77**:71–94.
13. Sommer RJ, Carta LK, Kim S-Y, Sternberg PW: **Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda, Neodiplogastridae).** *Fundam Appl Nematology*, in press.
14. Ferguson EL, Sternberg PW, Horvitz HR: **A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*.** *Nature* 1987, **326**:259–267.
15. Lewis EB: **A gene complex controlling segmentation in *Drosophila*.** *Nature* 1978, **276**:565–570.
16. Sulston JE, White JG: **Regulation and cell autonomy during postembryonic development in *Caenorhabditis elegans*.** *Dev Biol* 1980, **78**:577–597.
17. Sternberg PW, Horvitz HR: **The combined action of two intercellular signaling pathways specifies three cell fates during vulval induction in *C. elegans*.** *Cell* 1989, **58**:679–693.
18. Ferguson EL, Horvitz HR: **Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*.** *Genetics* 1985, **110**:17–72.
19. Ellis RE, Jacobson D, Horvitz HR: **Mechanisms and functions of cell death.** *Annu Rev Cell Biol* 1991, **7**:663–698.
20. Avery L, Horvitz HR: **A cell that dies during wild-type *C. elegans* development can function as a neuron in a *ced-3* mutant.** *Cell* 1987, **51**:1071–1078.